Aim 55

Southern blotting of DNA Fragments from Agarose Gel

Introduction

A technique which is used for transferring DNA molecules from agarose gel to solid support such as nitro cellulose filter or nylon membrane is termed as southern blotting. Presence of alkali helps for the denaturation of gel. DNA fragments get separated into two polynucleotide chains which can then hybridise with the probe.

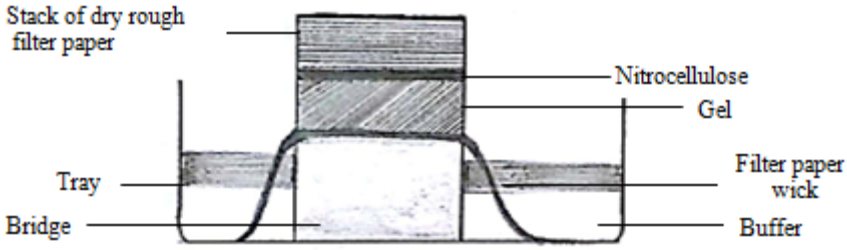
Membrane should be placed at the top of the gel. DNA molecules moves in upward direction and get attach to the membrane. Binding of DNA molecules to membrane can occur on exposure to UV light (1-2 min) or heat at 80°C for 2 h.

Requirements

1. Nylon membrane
2. Neutralizing solution (0.5 M tris- HCl buffer containing 1.5 M NaCl and 1 mM EDTA, pH 7.2)
3. Whatmann paper 3 mm
4. Denaturating solution (1.5 M NaCl solution containing 0.25 M NaOH)
5. Agarose gel with DNA fragments
6. SSC solution (10 x) {1.5 M NaCl containing 0.15 M sodium citrate, pH 7.0}
7. Rough filter paper
8. SSC solution (2X) {dilute 10X solution five times}
9. Glass tray and glass plate

Procedure

1. The DNA is denatured on gel by immersing the gel in denaturing solution for 30 min.
2. Discard the denaturating solution and rinse with distilled water.
3. Neutralizing solution is added and mixed softly.
4. Remove the above solution after 30 min and wash the gel with SSC solution.
5. Put some block in the centre of glass tray, place clean glass plate on the block.



6. Take Whatman's 3 mm paper and cut it into proper size so that its two ends dip in the buffer in tray. Put two layer of this paper on glass plate.
7. Pour SSC 10X solution in tray to fill it half.
8. SSC 10X solution is used to wet the Whatman's paper.
9. Take another Whatman's paper and cut it to the size equal to gel, wet with SSC 10X solution and place it on bridge.
10. Keep the gel on the top of the wet filter paper.
11. Take nylon membrane, cut it to size equal to the gel and wet the membrane with SSC 2X then place it on gel.
12. Take two pieces of Whatman's filter paper whose size is equal to gel, wet them with SSC 2X solution and leave

them over the membrane. No air bubble is entrapped between the papers.

13. Keep a stock of dry rough filter papers, cut them in size equal to gel on the top.
14. Place about 0.5 kg weight over it.
15. Due to capillary action buffer moves from the bottom filter paper through the gel, carrying the denatured DNA present in the gel. In nylon membrane as the buffer pass, DNA gets trapped in it. Keep the set undisturbed overnight.
16. From the top of the membrane remove the weight, stock of rough filter paper, whatmann filter paper.
17. Take out the membrane, wash it with SSC 2X solution then expose it to UV light to achieve the binding of DNA to the membrane for 1 – 2 min.
18. Hybrdisation can be done by this.

Precautions

1. Gloves should be used during work.
2. Care should be taken that there should not be any air bubble entrapped between Whatman's paper, gel and membrane.